

Pages 7
Code 1
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BEHAVIOUR OF PLANARIA IN INSTRUMENTAL LEARNING PARADIGMS

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In earlier studies on maze learning in planaria by myself and Rubinstein, which have already been reported (1962), we found evidence that not only were planaria capable of instrumental conditioning but seemed to manifest complex modes of ancillary behaviour resembling those found in higher vertebrates. Planarians trained in a two-choice maze situation using water withdrawal as the incentive, water restoration as the reinforcement, and light-dark cues randomized with respect to position initially showed no preference for one alternative over another. By the second session they showed an enhanced preference for the reinforced alternative whether that was the lighted or darkened side. Some continued to increase their preference for the reinforced alternative into the third or even fourth session. Following the session in which any given worm exhibited its maximum preference for the reinforced alternative, it did significantly worse than chance. A session or two following this inversion in choice behaviour, it would act lethargic and refuse to run the maze even though showing normal activity in the home bowl both immediately before and after its session in the maze. This effect was not due to accrual of chemical contaminants.

The planarians also manifested what appeared to be an analogue to "VTE" and "conflict" behaviour at around the end of the first session or beginning of the second, i.e. at about the point where it would appropriately be manifested in a higher animal such as a rat.

The inversion in choice behaviour and subsequent lethargy could be alleviated considerably by providing the worms with a larger chamber into which they could crawl during the inter-trial period without changing the fundamental character of the discrimination task. Refusal of fasted worms to eat in the confined space of the maze wells, and the readiness with which they would escape from the confines of the maze wells if given the chance, provided additional clues that this confinement was itself an aversive situation.

If the worms were provided with a route of entry and egress to the maze well system and

allowed to enter under their own volition, then they would eat in the maze wells. If allowed a prehabituating period in the chamber maze they exhibited a shorter latency of feeding than worms fasted the same length of time that had not been prehabituating (Best & Rubinstein, 1962b).

Are these patterns of behaviour isomorphic with those of the so-called emotionality syndrome seen in higher animals placed in a novel imprisoning environment? One can make another test. If the feeding response delay is an index of an emotional kind of state in the planaria as it is usually considered to be in higher animals (Hall, 1934) and the novel environment is made manifestly dangerous by, for instance, shocking the worm in it, then the feeding response should be delayed even more in that environment, i.e. one can see whether one can produce a "conditioned emotional response with stimulus generalization". This was done and the feeding delay was found to be greatly enhanced.

In the operant conditioning paradigm designed by Lee (1963) planarians are individually contained in small cylindrical lucite wells approximately 20 mm. in diameter and 12 mm. deep. A beam of light about a millimetre in diameter passes up through the transparent floor of the chamber and is detected by a small photodiode positioned above the chamber, with its photosensitive element aimed downward and shielded except for a small aperture. A large incandescent bulb illuminating the chamber from above provides aversive stimulation. Interception of the small detector beam is the response and 15 minutes of darkness the reinforcement.

Subjects are run in pairs, the two chambers being positioned side by side under the same stimulus light and hence equally illuminated by it. One of the members of the pair, for reasons apparent in a moment, is called the *experimental*. The other is called the *control*.

Experimental and control chambers, as well as their inmates, are the same except for the difference that resides in the contingencies of the programming circuits associated with the two chambers. If the experimental worm intercepts

its detector beam then the stimulus light is turned off for 15 minutes while interception by the control is ineffectual. Interceptions by experimental and control are automatically recorded on separate channels of an event recorder along with the state of the stimulus light. The pattern of stimulation impinging on the experimental and control is thus identical, except that there exists no contingency between what the control does and what happens to it.

One difference between our apparatus and Lee's is worth mentioning. The detector beam in Lee's chamber was positioned so that it passed at the perimeter of the circular floor while ours passed through at the centre. As the worms tend to travel around the perimeter, a higher natural operant level of response is to be anticipated in Lee's set-up than in ours. Repeating Lee's experiment on *Cura foremanii* we found essentially what he found except for the lower operant rates to be anticipated due to the modification just mentioned. Using 14 pairs of worms run continuously for periods ranging

from 42.5 to 191 hours we found for the percent of half-hour intervals containing either 1 or 2 responses an overall average of 30.9 for the experimentals against 15.1 for the controls. In the first 9 hours both experimentals and controls gave the same average of 22.8 per cent. on this measure. The mean cumulative response curves for experimentals and controls yield a picture consistent with the above. The two curves are identical for the first 9 hours and then gradually depart with experimentals higher than controls.

It has been suggested by Halas (1963) and in this meeting by Jensen that a more likely interpretation of Lee's results would be the following mechanism:

Planaria tend to move when the light is on and stop when it is off. Since the light goes off when the experimental intercepts the detector beam, the experimental is stopped in a closer proximity to the beam than the control and hence is more likely to be in a position to trigger it again.

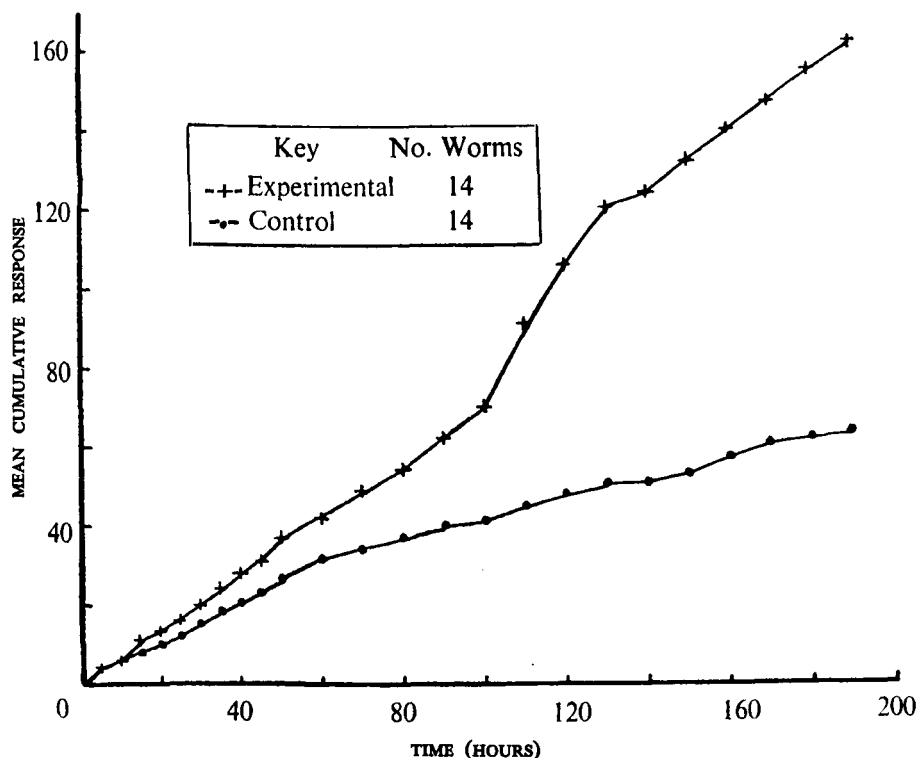


Fig. 1. Mean cumulative response curves of planaria (*Cura foremanii*) in operant conditioning paradigm using 15 minutes of darkness as reinforcement. Portion of curves beyond 120 hours based on only 3 experimental-control pairs.

Actually the planarians do not either start or stop right away when the stimulus light goes on or off, and this lag is of such length that they may go around the chamber half-a-dozen times or more after intercepting the detector beam. In the first 9 hours, a period of much longer duration than those on which the old classical behaviour observations on planaria are based (Hyman, 1951), both experimental and control give the same response patterns and both respond in the dark period of reinforcement and in the light. That there is a large random element in the response patterns of both is indicated by the fact that the interresponse times of both in this period is very nearly that for a temporal Poisson process.

A second alternative mechanism of the difference between experimentals and controls might reside in the following mechanism which we thought of. I can't remember whether this one was suggested by Halas or Jensen:

The photodiode is going to give some shadow. When the stimulus light is on one might imagine a natural disposition of the worms to head for shadow. If the experimental gets there first, then it is going to be more apt to intercept the detector beam and shut off the stimulus light. In this case there won't be a shadow for the control to head for, thus yielding a higher response rate and higher proportion of half-hour intervals containing one or two responses by the experimental.

This mechanism can be set into operation by appropriate manipulation of the experimental variables. If one paints a black spot under the photodiode right around the point in the floor where the detector beam passes through, paints the rest of the chamber white, and increases the stimulus light intensity, then one does, in fact, obtain a higher rate for naive experimentals over naive controls. But, under the circumstances of the experimental set-up used here, this is not the case, and for the first 9 hours, as already mentioned, the fact that the rate and pattern of experimentals and controls were the same precludes the possibility that such a mechanism is playing a role.

Darryl Pirok and I then went on to do a different experiment using the species of planarian *Dugesia dorotocephala* in this paradigm. Instead of running worms continuously for the entire period they were put into the apparatus for an initial habituation of 1 hour without stimulus light or detector beam. Both detector beam and stimulus light were then turned on for a period

of 7 hours. At the end of this 7 hours the stimulus light was turned off to allow a terminal de-habituation of 1 hour at the end of which the worms were removed and placed in individual fingerbowls in a dark cupboard for 39 hours. They were then put back into their respective chambers for one hour's habituation, as before, and the lights again turned on for a second work test session of 7 hours.

By adding up the hours it can be seen that the worms are thus administered a 7-hour session and then a second 7-hour session at the same time of day 2 days later.

For the moment I want to consider the results on all of our experiments except those in which the second session fell within plus or minus 2 days of the time of the half-moon. I'll return to elaborate on this cryptic remark a little later. Those falling outside of this half-moon time will be designated as "Phase I". Those falling inside this half-moon time will be designated "Phase II". Thus, for the moment, I wish only to consider the results for Phase I subjects.

Mean cumulative response curves for experimentals and controls do not show any evidence of differing from one another during the first 7-hour session. In the second 7-hour session, however, they are of different slope right from the onset, with that for experimentals higher than that for the controls. These results are shown in Fig. 2. There are 64 pairs of Phase I subjects. Since there was no difference between Phase I and II subjects in the first session the first session data for all the subjects in both phases have been pooled. Vertical bars show standard deviation. This means that sometimes between the close of the first session, in which they gave no evidence of learning, and the beginning of the second (a period in which there was no opportunity for contact with the training situation) they learned it, insofar as the difference in rate of responding between experimental and control can be considered to be a measure of learning. I believe that it is.

If a human subject is given an intensive training session on a task of such difficulty that he has learned it only imperfectly by the end of the session, then given a time-out of proper duration, he will, upon retest, exhibit an improved performance. In other words he seems to have learned during a period of no overt practice. Human beings are known to be introspective animals and so it was thought that this kind of learning stemmed from some kind of

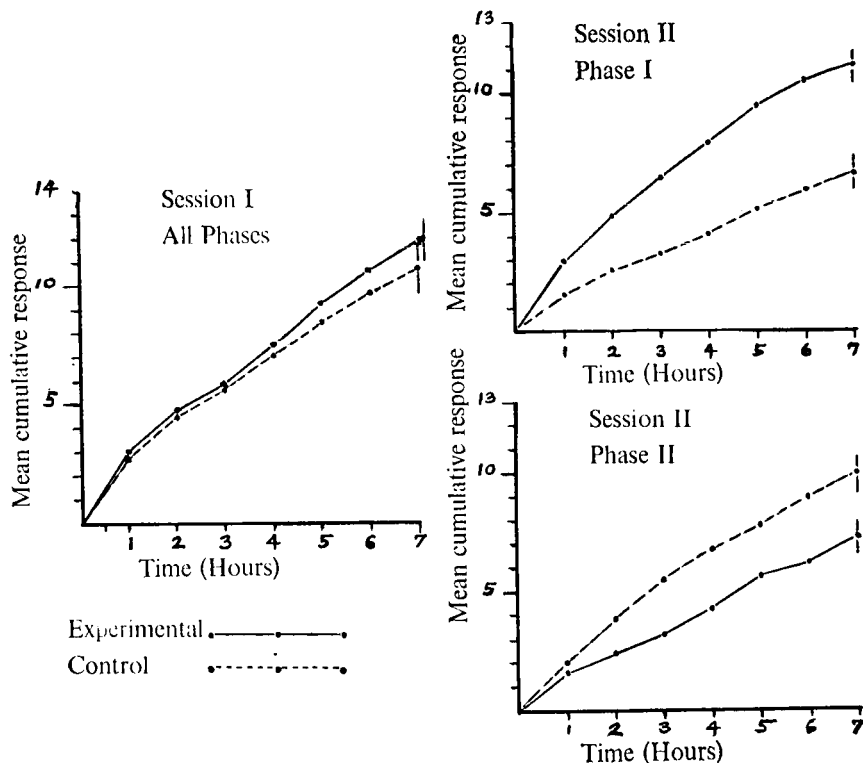


Fig. 2. "Reminiscence" development of an operant response by the planarian *Dugesia dorotocephala*. Mean cumulative response curves of experimentals and stimulus-yoked controls for session 1 and 2. Forty-one hours between end of session 1 and beginning of 2. Phase I curves based on 64 pairs of subjects, Phase II curves on 68 pairs. Vertical bars show standard deviation of the mean.

thinking back over the problem, the phenomenon was called the "reminiscence effect". It was, however, also observed subsequently in rats (Bunch & Magdick, 1933; Buxton, 1942).

Because the effect observed here in the planarians seems to be phenomenologically similar to that observed previously in mammals I will refer to it in the same way as my predecessors and simply call it *reminiscence effect*. I would not wish to contend that the planarians actually learn that sticking their heads in the detector beam turns off the stimulus light. There is a considerable component of what seems to be random behaviour in the response patterns of both experimentals and controls even at the point at which the two differ significantly. It may be that all the experimentals are learning is something to the effect that "one fares better if one moves" and all the controls are learning is that "it doesn't do any good to move". But if one actually watches a rat in a skinner box, one sees

behaviour that makes one doubt whether the rat really knows that depressing the lever is what delivers the reinforcement. The 41-hour interval for the present reminiscence effect development in planaria compares rather closely with that found for motor task learning in humans.

Considering that the mammalian brain has 10^{10} to 10^{11} neural elements while the planarian nervous system has only about 10^3 , a difference of some 10^7 to 10^8 fold, it is interesting that the reminiscence development times should be of the same order of magnitude. In view of this, it is difficult to consider the reminiscence development time as the time required for a reshuffling of neural circuits into some kind of optimal McCulloch & Pitts (1943) Boolean network. My own tendency is to regard this as the time required for the biochemical synthesis of the memory stuff.

Whether the planarians are really "thinking

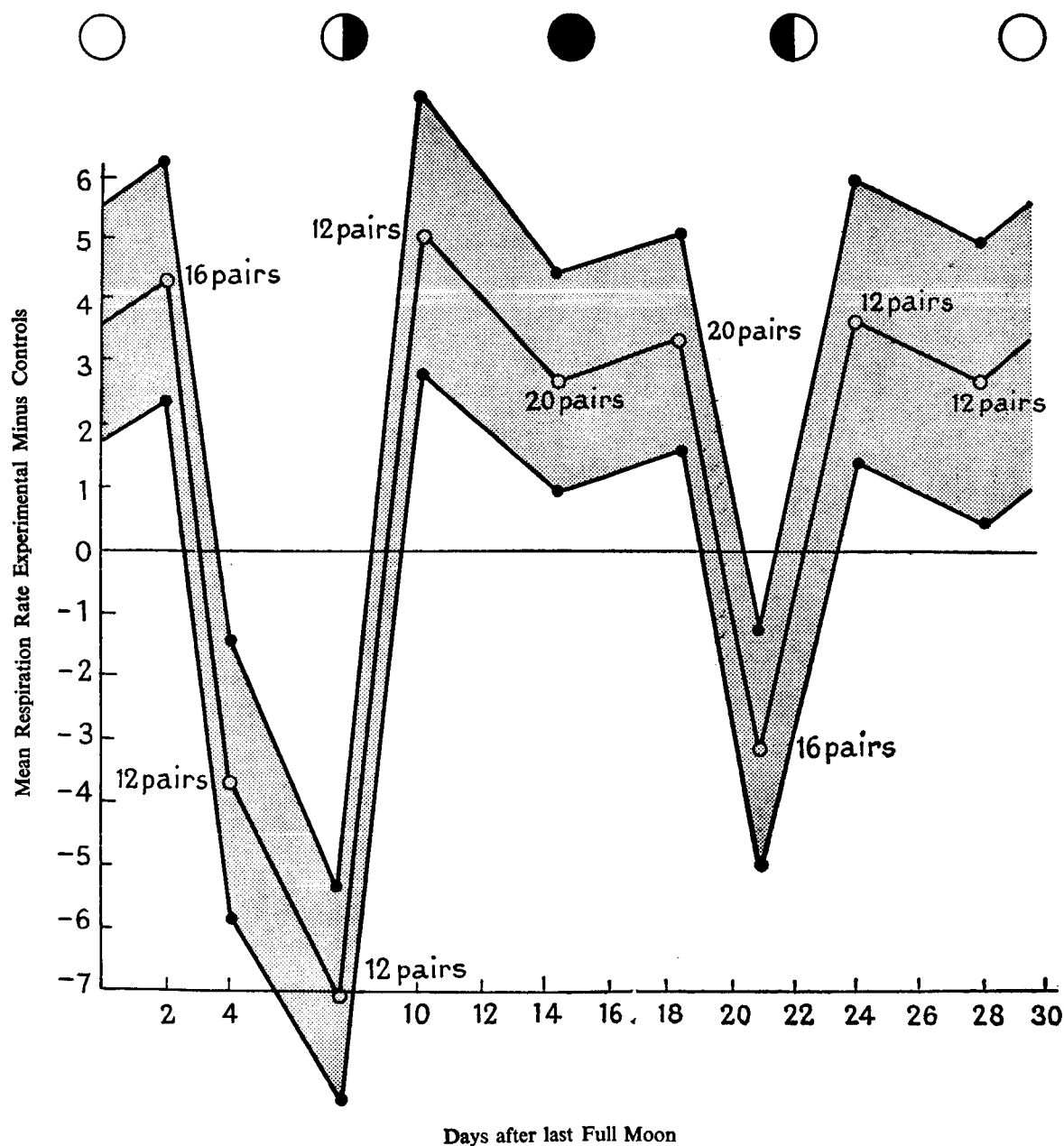


Fig. 3. Semi-lunar cycle in "reminiscence" development by the planarian *Dugesia dorotocephala*. Mean response difference of experimentals and controls in second session according to time of lunar month in which experiment was performed. 41-hour separation between first and second session. Based on 132 pairs of subjects run over 6 months period beginning October 1st, 1963. Shaded area denotes region lying between limits set by standard deviation of mean. Number of pairs on which each point is based is indicated.

things over" is a question I'll leave for the more philosophically inclined to ponder.

The curious remark made a little earlier concerning the lunar phases needs elaborating at this point. For the 132 pairs sampled from our laboratory colony of *Dugesia dorotocephalia* and tested in the reminiscence paradigm over the 6 month period beginning about October 1st, 1963, there appears to be a cycle of very nearly semi-lunar, i.e. Ca. 15 days, period in manifestation of this reminiscence effect. The difference between the response rates of experimentals and controls in the second session can be considered, in view of the foregoing discussion, to be a measure of the reminiscence effect. Fig. 3 shown the fluctuation of this measure as a function of the time in the lunar month on which the second session fell. The time separating the first and second sessions is constant throughout.

Fig. 3 is a plot of the average response difference between experimentals and controls in the second session against the number of days following the last full moon on which the second session date fell. The upper and lower bounds show the standard deviations of the means.

The χ^2 of this set of points against the zero line is 29.7 with 9 degrees of freedom which gives $p < 0.001$. Since we have not applied the power spectrum analysis method of Blackman & Tukey (1958), one can't say yet whether this periodicity is exactly 15 days or whether there are other harmonic components of fractional or integral multiple periods that are summing to give the 15 days cycle.

It is reasonable to ask what prompted an examination for a cycle of this period. In the period of 6 months ranging through the late summer of 1962 into the first part of 1963 Corwin Dunn and I tested planarians of the species *Cura foremanii* in a maze involving 3 sequential T junctures. The subjects were given a session each day. Each session entailed two complete passages through the maze. A criterion session was one with no mistakes. It was found that the subjects tended to produce criterion sessions within a few days of one another irrespective of the number of training sessions they had had. It was found that these criterion session times tended to recur within a period approximating a lunar month and to slightly lag the time of the full moon. Since the planarians were kept in an air-conditioned laboratory and had no opportunity to see the moon we were, for fairly obvious reasons, reluctant to publish these results without further evidence. It did however, alert

us to the possibility of cycles of such periodicity.

In the reminiscence paradigm there is no evidence of any such cyclic variation in the differences between response rates of experimentals and controls for the first session.

The laboratory at Colorado State University, in which these operant conditioning experiments were performed, is a mile high. This means that the background radiation from cosmic rays and "solar wind" bombardment is several fold that at sea-level and these do exhibit more or less regular periods of fluctuation correlated with the astronomical periods. In view of the recent finding of Garcia *et al.* (1964) that animals are capable of direct detection of low levels of X-irradiation it seems possible that there may be an endogenous periodicity in planaria of approximately 15 days which becomes synchronized to the astronomical period by the clues provided by cosmic ray background fluctuations.

Or one might reason as follows. Most of the endogenous biological rhythms, i.e. biological clocks, are not very precise in the free-running situation where external periodic reference cues are missing. They are, however, usually within about 10 per cent. of the period to which they are normally synchronized in nature. In our laboratory our large colony pans usually contain about 900 to 1600 individuals. If there are social interaction effects, and we have evidence that there are, then one might anticipate a tendency to synchronize their periods. Such an interaction would thus tend to pace the periods of the individual planaria with the average of the colony. The average for the colony would, however, have a much smaller variability or margin of error than that for individuals maintained in isolation. Thus, if the individual error is 10 per cent., one might expect the colony error to be only about 0.3 per cent. In the 2 years of maintenance in the moonless conditions of the laboratory there would have been about 48 of these semi-lunar cycles. The colony error in reckoning the phase of the moon at the end of this time might, by this token, still be only about 1/10 to 2/10 of a cycle, an error of about 1½ to 3 days.

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*This work was supported in part by National Aeronautics and Space Administration Grant NsG 625 and in part by National Institutes of Health Grant MH 076203-02 EP R01.

Summary of discussion of the paper

BEHAVIOUR OF PLANARIANS IN INSTRUMENTAL LEARNING PARADIGMS

By J. B. Best

General Discussion

In reply to criticism by Davenport, Best claimed that in his experiments the response rate gave some measure of the general level of activity.

Thorpe asked what the phrase "home bowl" meant for a planarian, and how such a bowl was recognised. If it is true that planarians can in fact recognise a home, then this suggests that they are capable of very rapid learning. Best said that he did not know how planarians did this, but they certainly seemed to have some perception of the space in which they lived. They will not at first eat in a small new bowl and they will eat sooner in a large than a small new bowl. This may be a general feature in the behaviour of many animals. For instance, Ratner keeps his annelid worms in a circular tube so that they can move round and round without being trapped. If they are in a blind ended tube they rush to the open end and keep moving back and forth and become so upset that they cannot be used in experiments.

Best then went on to describe the behaviour of *Dugesia* in relation to mazes. If you place this planarian in a new maze then it keeps raising its head out of the water. This is a behaviour pattern only shown when it is "unhappy". In a new maze full of water only one out of eleven animals eat after five days. but if the maze is put into the planarians living bowl and they are allowed to go in and out as they wish, then food will be taken almost immediately in the new maze.

Jensen pointed out that this all went to show that handling animals was of vital importance in the experimental technique used in any particular study. Best then added that the picture was not as simple as all that, since if you handle a planarian and transfer it to a large bowl and not a small one, then it will eat within half an hour if it has been previously starved, but will not eat for many days if the transfer has been to a small bowl. The chairman suggested that perhaps these animals suffered from claustrophobia.